

T-strains of mycoplasma and nongonococcal urethritis

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Since the isolation by Dienes and Esdall (1937) of a strain of *Mycoplasma* from a patient with a Bartholin's gland abscess, numerous attempts have been made to provide definite proof of the aetiological role of *Mycoplasma* in urogenital tract infections, especially nongonococcal urethritis (NGU). Most of the earlier studies were concerned with *Mycoplasma hominis*, but it now appears that this species is a normal inhabitant of the male urethra and not a cause of NGU (Taylor-Robinson, Addey, Hare, Jones, and Dunlop, 1969).

Shepard (1954) described a new member of the genus *Mycoplasma* which he called T-strain, and which he suggested might be associated aetiologically with NGU (Shepard, 1954, 1960). Since then numerous reports have appeared comparing the isolation rates of T-strains in cases of gonococcal urethritis (GU) and of NGU, and in healthy males. The results of these studies are summarized in Table I. In view of these findings from a variety of different

countries (America, England, Denmark, Australia, Canada), it was decided to examine the isolation rates of *Mycoplasma* from the urethras of males in Israel.

Material and methods

Urethral specimens were obtained from 96 males who attended the Dermatology out-patient clinic of the Hadassah-University Hospital. These patients were divided into three groups:

- (i) 52 patients with clinical signs of urethritis but in whom *N. gonorrhoeae* could not be identified microscopically or by cultural methods.
- (ii) 17 patients with culturally proven gonorrhoea.
- (iii) 27 subjects with no clinical history or signs of urethritis (healthy controls).

The average age of the patients in the first two groups was 30 years. In the third group the ages ranged from 20 to 70 years, and they were therefore subdivided into those aged 20 to 45 years (17 subjects) and those over 45 years (10 subjects).

The urethral discharges were collected on cotton-wool tipped swabs or with a sterile platinum wire loop. Material

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TABLE I Summary of previous investigations of T-strain mycoplasma in male urethra

Authors	Year	Country	Gonorrhoea		NGU		Controls	
			No.	Per cent. positive	No.	Per cent. positive	No.	Per cent. positive
Shepard	1956	USA	—	—	74	62	—	—
Shepard	1959	USA	—	—	650	70	—	—
Ford, Rasmussen, and Minken	1962	Canada	27	26	45	60	55	22
Ford and du Vernet	1963	Canada	—	—	100	79	200	34
Shepard, Alexander, Lunceford, and Campbell	1964	Philippines (US Navy)	139	13	64	52	—	—
Csonka, Williams, and Corse	1966	Gt Britain	50	28	101	70	—	—
Ingham and others	1966	Gt Britain	36	61	45	66	54	48
Black and Rasmussen	1968	Denmark	60	35	56	46	150	49
Shipley, Bowman, and O'Connor	1968	Australia	—	—	24	70	13	23
Fowler and Leeming	1969	Gt Britain	404	52	314	56	123	41
Dunlop, Hare, Jones, and Taylor-Robinson	1969	Gt Britain	—	—	30	63	—	—

NGU = Nongonococcal urethritis

from the normal controls was collected from the urethra with sterile wire loops. All specimens were immediately plated on Thayer-Martin medium* for the isolation of *Neisseria gonorrhoeae* and on the following media for *Mycoplasmas*:

(i) PPLO agar (Difco) containing 20 per cent. unheated horse serum, 10 per cent. yeast extract, 1/2000 thallium acetate, and 1000 units penicillin G per ml. (Chanock, Hayflick, and Barile, 1962).

(ii) Trypticase soya broth with ionagar No. 2 (Oxoid) containing 20 per cent. horse serum, 1000 units penicillin G per ml., 0.1 per cent. urea, and 0.002 per cent. phenol red (pH 6.0) (Shepard, 1969).

The inoculated plates were incubated for 2 to 4 days at 37° C., those for T-strains in an atmosphere of CO₂ in a candle jar.

Mycoplasmas producing normal-sized colonies were identified by means of specific growth-inhibition, using antisera prepared in rabbits according to the method of Clyde (1964). Antigens used for immunization were *M. hominis* type 1 (P621), *M. fermentans*, and a locally isolated strain of *M. hominis*. The colonies growing on Shepard's medium were identified as T-strains on the basis of their colonial morphology, and ability to metabolize urea.

Results

The colonies that developed on Chanock's medium were all identified as *M. hominis* type 1 by growth inhibition. Many of the isolates gave considerably larger zones of inhibition with the antiserum produced against the locally isolated strain of *M. hominis*

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than with that produced against the type strain. This tends to confirm the serological heterogeneity of *M. hominis* strains which has been described previously (Razin, 1968). *M. fermentans* was never isolated.

The T-strains usually grew after 24 to 48 hours, were urease positive, and had a well-defined brown colour, which is not seen in other *Mycoplasma* colonies. In some cases both *M. hominis* and T-strains were isolated on Shepard's medium (Figure).

The results as summarized in Table II show that T-strains were isolated in 65 per cent. and *M. hominis* in 17 per cent. of the patients with NGU; both species were isolated together from four patients. Ten of the seventeen patients with gonorrhoea (58 per cent.) yielded growth of T-strains and three (17 per cent.) of *M. hominis*. In the younger control group of seventeen men, T-strains were

TABLE II Summary of results of urethral cultures for mycoplasmas

Group	Age (yrs)	Total No. of cases	Number of cases with			
			T-strains		<i>M. hominis</i>	
			No.	Per cent.	No.	Per cent.
NGU	±30	52	34	65*	9	17*
GU	±30	17	10	58**	3	17**
Healthy Controls	20-45	17	8	47	0	
	>45	10	2	20	0	

NGU = nongonococcal urethritis

GU = gonococcal urethritis

*Four cases had both T-strains and *M. hominis*

**Two cases had both T-strains and *M. hominis*

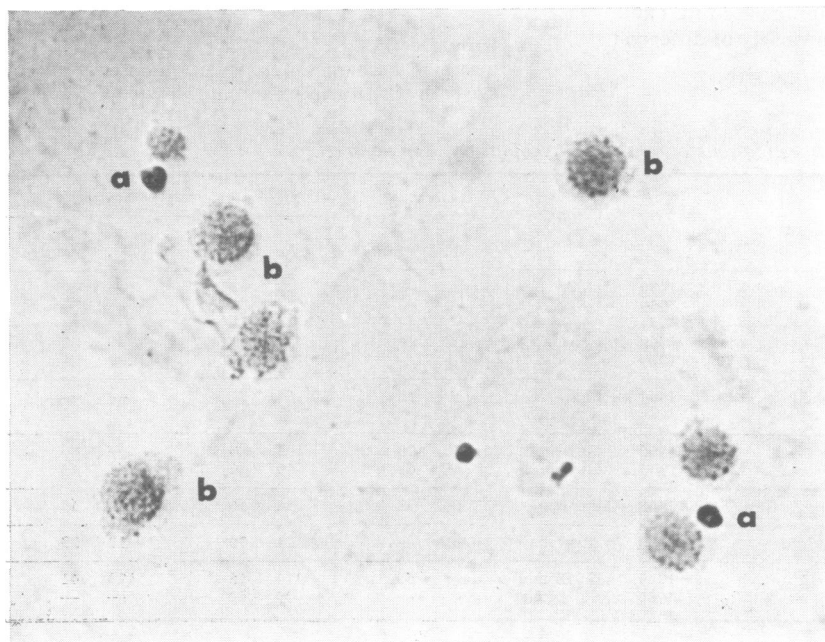


FIGURE Shepard's medium, showing colonies of T-strain (a), and *Mycoplasma hominis* (b)

isolated in eight (47 per cent.) and in the older group in only 20 per cent. *M. hominis* was not isolated from any of the symptomless controls.

Discussion

The number of cases in the present series is small and definite conclusions are probably not justifiable. Several interesting aspects, however, are worthy of comment. The isolation rates of T-strain mycoplasmas in gonorrhoea and in NGU were similar (58 and 65 per cent. respectively). This rate for NGU is similar to that found in the previous studies, while the rate for GU is higher than in most of the earlier series (Table I). The method used in this study to isolate the T-strains was very similar to that used by previous workers, except for the use of a simple candle-jar to provide raised CO₂ tension instead of the more usually recommended method of 20 per cent. CO₂ (Black and Rasmussen, 1968) or 20 per cent. CO₂ and 80 per cent. N₂ (Ford and MacDonald, 1963; Ingham, MacFarlane, Hale, Selkon, and Codd, 1966). It would appear from our results that the simpler method of raising CO₂ tension is not less effective than those previously described.

The similar isolation rates for T-strains in patients with GU and those with NGU suggest that these organisms do not play an aetiological role in the latter condition. The higher rates in the patients with urethritis than in those without may indicate an increased susceptibility of the inflamed mucosa to colonization by T-strain mycoplasmas.

The lower incidence of T-strains in the older group of healthy controls suggests that the mucosa of young males may be more susceptible to colonization than that of older men. Black and Rasmussen (1968) found T-strains in 25 per cent. of a group of physicians aged 29 to 48 years, but in 48 per cent. of those in the three other control groups whose average age was 24 years. Another explanation might be the higher rate of sexual activity in younger men, with more exposure to potential female carriers of the organism.

The low isolation rates for *M. hominis* in this series of patients, all of whom had been circumcised, is consistent with the finding of Hare, Dunlop, and Taylor-Robinson (1969) that *M. hominis* was present in 49 per cent. of uncircumcised males and in only 23 per cent. of those circumcised. They could find no such difference in the isolation rates of T-strains in circumcised and uncircumcised males, and suggested that *M. hominis* may inhabit the preputial sac as well as the urethra.

The considerable variation in isolation rates of T-strains from cases of NGU described in the literature is reflected in the varying opinions as to their aetio-

logical significance. Fowler and Leeming (1969), on the basis of the largest single series of cases investigated, concluded that there was no causal relationship between T-strains and NGU. Similar conclusions were reached by Black and Rasmussen (1968), by Ingham and others (1966), and by Hare and others (1969), although Shepard (1970) still believes that there is strong evidence incriminating the T-strain as one of the major causes of NGU. The present modest series adds additional support to the suggestion that there is no aetiological relationship between T-strains and NGU.

Summary

T-strain mycoplasmas were isolated from the urethras of 65 per cent. of 52 males with NGU, from 58 per cent. of seventeen males with gonorrhoea, and from 47 per cent. of healthy males aged 20 to 45 years. Only two of ten healthy males over the age of 45 years harboured T-strains in their urethras.

M. hominis was relatively infrequent in this circumcised population, and was not isolated from any of the healthy controls and from only twelve of the 69 males with urethritis.

T-strain mycoplasmas do not seem to play a causative role in NGU.

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Souches T de mycoplasmes et l'urétrite non gonococcique

SOMMAIRE

Des mycoplasmes souche T ont été trouvés dans 65 pour

cent des urèthres de 52 hommes atteints d'urétrite non gonococcique, dans 58 pour cent de 17 hommes atteints de gonococcie et dans 47 pour cent d'hommes sains, âgés de 20 à 45 ans. Deux seulement des 10 hommes sains de plus de 45 ans hébergeaient des souches T dans l'urètre.

M. hominis fut relativement rare chez les circoncis, il ne fut pas trouvé chez les témoins sains, et chez 12 seulement des 60 hommes présentant une urétrite.

Les mycoplasmes de souche T ne semblent pas jouer un rôle étiologique dans l'urétrite non gonococcique.